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REMARKS

Status Summary

Claims 1-96 are pending. Claims 1-73, 80, and 88 are withdrawn from consideration as being directed to a non-elected species. Claims 74-79, 81-87, and 89-96 were examined with the species of an anti-CD20 antibody. Claims 81-82, 84, 86, and 90 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to enable practice of the invention based on perceived nonavailability / nonreproducibility of the RITUXAN® antibody. Claims 74-79, 81-87, and 89-96 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Alas et al. (1998) *Blood* 92:601a in view of Levy et al. (1994) *J Clin Invest* 93:424-428 and U.S. Patent No. 6,183,744 to Goldenberg et al.

New claims 97-102 are added. Reconsideration in view of the new claims and following remarks is respectfully requested.

Rejection of Claims Under 35 U.S.C. § 112, First Paragraph

Claims 81-82, 84, 86, and 90 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabling practice of the invention. The examiner contends that it is uncertain whether the RITUXAN® (rituximab) antibody is known and publicly available and/or reproducible from the written description. Official action, page 3, item 7. This rejection is respectfully traversed.

Applicant respond that cells expressing the RITUXAN® (rituximab) antibody are publicly available as deposit number 69119 from the American Type Culture Collection. Further to the previously provide receipt of deposit, enclosed herewith is a letter from the ATCC dated January 7, 2004, which confirms that deposit number 69119 is publicly available. A representative of the ATCC further acknowledged via telephone that the inability to identify deposit number 69119 through their online catalog was a defect to be corrected by the ATCC.

In addition, the amino acid sequence of RITUXAN® (rituximab) is disclosed in U.S. Patent No. 5,736,137, which issued on April 7, 1998. Contrary to the examiner's assertion, the complete nucleotide sequence of the ATCC antibody is disclosed in the '137 patent. *See* Figures 3A-3F and SEQ ID NO:3, which provide the complete nucleotide and amino acid sequence of the C2B8 antibody in the TCAE vector. Applicant further submits that a skilled artisan could readily prepare anti-CD20 antibodies useful in the present invention given the

sequences of the heavy chain and light chain variable regions, as set for in Figures 4 and 5 of the '137 patent.

Based on the foregoing, this rejection of claims is believed to be rendered moot, and withdrawal of the rejection of claims 81-82, 84, 86, and 90 under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Rejection of Claims Under 35 U.S.C. § 103(a)
Based on Alas in view of Levy and Goldenberg

Claims 74-79, 81-87, and 89-96 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Alas et al. (1998) *Blood* 92:601A (Alas) in view of Levy et al. (1994) *J Clin Invest* 93:424-428 (Levy) and U.S. Patent No. 6,183,744 (Goldenberg). Official action, page 3, item 8. This rejection is respectfully traversed based on the arguments set forth below.

The examiner bears the burden of presenting a *prima facie* case for obviousness, with a showing of such *prima facie* obviousness requiring: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) the teaching or suggestion of all the claim limitations of the applicant's invention in the combined references; and (3) a reasonable expectation of success. MPEP § 2143.

Alas teaches that the C2B8 antibody can be used to sensitize B cell lymphoma cells to cytotoxic agents. Alas observed that cells treated with C2B8 downregulate the expression of IL10. Based thereon, Alas postulated that the C2B8 antibody is effective based on regulation of IL10, which in turn regulates cellular apoptotic proteins. Levy teaches that IL10 enhances cell viability by inducing bcl-2 expression. This protective effect is abolished on addition of an anti-IL10 antibody. The use of an anti-IL10 antibody in Levy is limited to an IL10-induced cell protection. The examiner relies on Goldenberg as teaching multimodal therapies. In the view of the examiner, it would have been obvious to combine an anti-CD20 antibody with an anti-IL10 antibody because removal of IL10 abolishes the protective effects of bcl-2. Advisory action dated September 17, 2003, page 2.

The examiner has not established a *prima facie* case of obviousness because the Goldenberg reference, as well as other teachings in the art as of the filing date of the present application, *teach away* from the claimed combination therapy. Thus, the cited documents fail to teach, suggest, or motivate the claimed invention, and a skilled artisan would not expect to perform the claimed invention with any reasonable chance of success.

As stated previously, at the time of the instant invention, the use of IL10 antagonists in cancer therapy was controversial. The literature contained reports that both supported and discounted a correlation between cytokines and disease progression. The examiner has cursorily dismissed references that are clearly contrary to the examiner's position, and applicant respectfully requests that the examiner substantively consider the teachings of these documents.

With respect to the noted uncertainty in the art, the examiner has responded merely that "Bonnefoix has been addressed previously." Advisory action, dated September 17, 2003, page 2. The examiner previously stated that "Bonnefoix et al does not teach any IL10 antagonists and it is clear from the prior art in the rejection cited that IL10 is important for cancer therapy and regulates bcl-2." Official action, dated July 8, 2003, page 8, item 8. Bonnefoix et al. (1997) *Leuk Lymphoma* 25:169-178 (Bonnefoix) found that cytokines, including IL10, could either inhibit or stimulate proliferation of lymphoma cells of various histological subtypes. Applicant respectfully submits that an absence of teaching IL10 antagonists does not preclude the relevance of the reference. Based on Bonnefoix, which constitutes knowledge in the art prior to filing of the present application, it was unclear whether IL10 or an IL10 antagonist could be useful for cancer therapy.

Further with respect to the uncertainty in the art as to modulation of IL10 for cancer therapy, the examiner states that "Goldenburg teach multimodal therapy with anti-CD22 and chemotherapy. It is unclear how this teaches away from the invention." Advisory action, dated September 17, 2003, page 2. In addition to teaching use of an antibody that recognizes a B cell antigen, *i.e.* anti-CD22, in combination with chemotherapy, Goldenberg also describes treatment of B cell malignancies using an anti-CD22 antibody in combination with cytokines, such as IL10 (claim 15). Thus, Goldenberg teaches multimodal cancer therapies that include IL10, which is *directly opposite* to the claimed use of a multimodal therapy premised on inhibition of IL10. Although the examiner has relied on Goldenberg for the limited teaching of multimodal cancer therapies, it is improper to disregard the teaching of Goldenberg with respect to particular multimodal therapies related to use of IL10, which are most relevant to, albeit contrary to, the instant claims.

In addition, the examiner has not responded to applicant's previously submitted arguments with respect to U.S. Patent No. 5,770,190 to Bruserud (Bruserud). Similar to the methods of Goldenberg, Bruserud teaches that administration of IL10 (*not* IL10 antagonists as

presently claimed), optionally in conjunction with chemotherapeutic agents, for treatment of acute leukemia. Specifically, claim 1 of the '190 patent is directed to "[a] method for treating an acute leukemia in a mammal, comprising administering a therapeutically effective amount of interleukin-10 to said mammal."

Thus, the teachings of Alas, Levy, and Goldenberg, when considered alone or in combination, fail to motivate the use of an anti-CD20 antibody in combination with IL10 antagonists as recited in the instant claims. Further, the teaching of Goldenberg *teaches away* from the present invention. Similar references, including Bonnefoix, Goldenberg, and Bruserud, also *teach away* from use of IL10 antagonists for treatment of B cell malignancies, as now claimed.

Based on the foregoing arguments, applicant believes that claims 74, 76-79, and 83 are unobvious over the cited references in accordance with 35 U.S.C. § 103(a). Claims 75, 81-82, 84-87, and 89-96 ultimately depend from claims 74, 76-79, and 83 and are therefore also believed to be patentably over the cited references. Thus, applicant respectfully requests that the rejection of claims 74-79, 81-87, and 89-96 under § 103(a) be withdrawn.

Discussion of New Claims

New claims 97-102 are added, which specify use in the claimed methods of an anti-CD20 antibody comprising a heavy chain variable region of a C2B8 antibody, and a light chain variable region of a C2B8 antibody. Support for the new claims can be found in the originally filed specification, including at page 24, line 5, through page 25, line 5, wherein use of chimeric antibodies, such as antibodies having human constant domains and other humanized antibodies, is described; and at page 25, lines 20-21, wherein use of the C2B8 antibody is described.

At the time of filing the present application, a skilled artisan could prepare chimeric and humanized anti-CD20 antibodies with human effector functions according to the invention and without undue experimentation. *See e.g.*, Ruth D. Mayforth (1993) Designing Antibodies, Academic Press, Inc. San Diego, pp. 91-92 (copy enclosed). This text, available prior to the filing date of the instant application, summarizes genetic engineering approaches for preparing chimeric antibodies that include non-human variable regions and human constant regions. Mayforth also describes that constructs encoding chimeric antibodies can be modified to introduce mutations in the variable region gene segments that alter the binding affinity of the chimeric antibody for its target antigen. Thus, using standard molecular

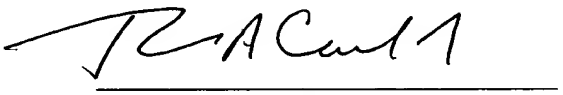
biology cloning techniques, one could readily prepare anti-CD20 antibodies of the invention based on the disclosed sequences of anti-human CD20 variable regions and known sequences of antibody constant regions, including human constant regions.

Conclusion

All objections and rejections having been addressed, it is respectfully submitted that the present application is in condition for allowance and a Notice to that effect is earnestly solicited. If any points remain in issue, which the examiner feels may be best resolved through a personal or telephone interview, he is kindly requested to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,

PILLSBURY WINTHROP LLP



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Per our conversation, this is to confirm that the above referenced patent deposits assigned ATCC No. HB-11712 and ATCC No. 69119 have been released for public distribution and are currently available for order. If you need further information please contact me. Thank you.

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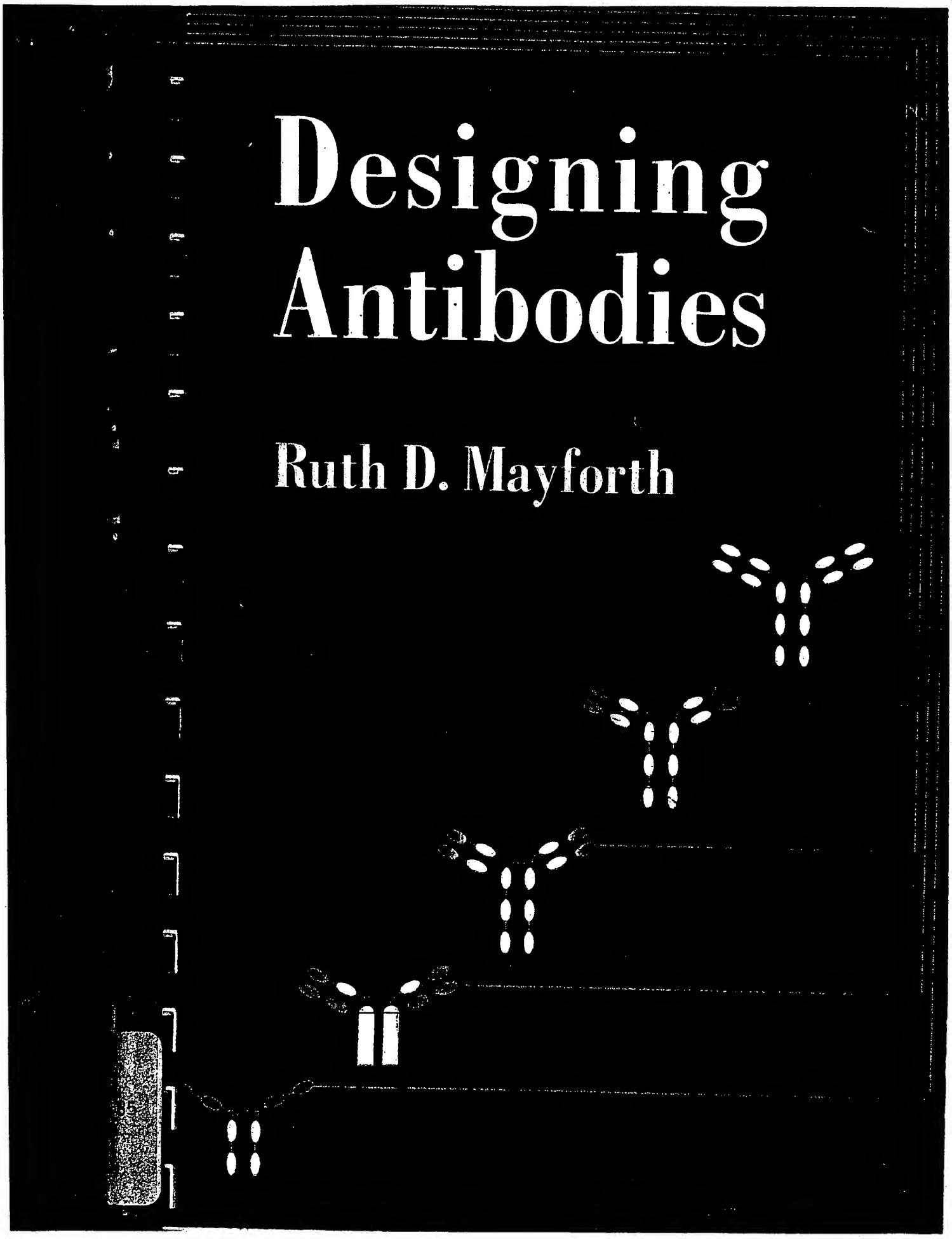
Designing Antibodies

Ruth D. Mayforth



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Plasmids are used to manipulate and transfect antibody genes. [Other vectors that are used in genetic engineering are phages (such as bacteriophage M13 and λ) and cosmids (which can incorporate very large stretches of DNA); these vectors are mainly used for making libraries.] Plasmids customarily contain sequences coding for selection markers, promoter/enhancer transcriptional control elements, origin of replications, splicing signals, and polyadenylation sites. Two frequently used bacterial selection markers are the *neo* gene and the *gpt* gene. The *neo* gene confers resistance to the antibiotic neomycin (G418). The *gpt* (xanthine-guanine phosphoribosyl transferase) gene confers resistance to mycophenolic acid. Transfectants that are selected in the presence of neomycin or mycophenolic acid have incorporated a plasmid expressing the *neo* gene or *gpt* gene, respectively (Lefranc and Lefranc, 1990).

Antibody genes can be modified in a variety of different ways. For instance, an entire exon can be spliced together with an exon (or exons) from a different antibody or another molecule. *Chimeric* antibodies are constructed by connecting the DNA that encodes the mouse variable region with exons that encode human constant regions (see Chapter 3). Likewise, exons encoding antibody genes can be linked to exons encoding toxins to create recombinant immunotoxins (see Chapter 4).

Alternatively, specific or random alterations can be introduced into the DNA sequence of a given antibody exon. Specific mutations are introduced through *oligonucleotide-mediated site-directed mutagenesis*, in which an oligonucleotide containing the desired alteration is synthesized and used as a primer to synthesize a gene that contains the mutated nucleotide or nucleotides (Kingsman and Kingsman, 1988). (As discussed extensively in Chapter 3, this technique has been used to humanize rodent antibodies, creating antibodies that have rodent hypervariable regions but are otherwise human. Briefly, oligonucleotide primers that contain the rodent hypervariable regions and small portions of the human framework regions are used to engraft the rodent CDRs onto human variable-region DNA.) Random alterations can also be introduced, producing an array of different mutants that can be screened and selected for the desired property. Various different techniques have been developed to introduce these random alterations. For instance, the vector carrying the gene of interest can be passaged through a strain of *Escherichia coli* (*E. coli mutD*) that has a 10^3 - to 10^5-fold higher frequency of spontaneous mutations than the wild-type strain (Fowler *et al.*, 1986). Alternatively, random mutations can be introduced through error-prone PCR (the polymerase chain reaction, to be described in detail shortly) (Leung *et al.*, 1989). In a more traditional approach, double-stranded DNA is nicked and digested with an endonuclease to generate a single-

istered together with the OKT3 antibody, a HAMA response of both anti-idiotypic and anti-isotypic antibodies eventually arose in a majority of the patients. The anti-idiotypic antibodies (rather than the anti-isotypic antibodies) neutralized the therapeutic effect of OKT3 by binding the variable regions of the OKT3 antibodies and directly preventing them from binding to the T cells (Chatenoud *et al.*, 1986; Jaffers *et al.*, 1986; Hirsch *et al.*, 1989).

Some measures have been useful in minimizing the HAMA response. As previously mentioned, simultaneous administration of immunosuppressive agents is helpful in reducing the magnitude of the response. Studies in mice have demonstrated that pretreatment with anti-CD4 antibodies inhibits the development of a humoral immune response to human, rat, rabbit, or hamster antibodies (Benjamin *et al.*, 1988; Hirsch *et al.*, 1989). Anti-CD4's efficacy at reducing the HAMA response in humans remains to be tested. Also, the HAMA response is dose dependent; thus, it may be possible to administer doses that are low enough to minimize the HAMA response yet still achieve a therapeutic effect.

In spite of these preventive measures, the HAMA response remains a significant hindrance to the use of rodent monoclonal antibodies in human therapy. In addition, some rodent isotypes are poor (or ineffective) at mediating human effector functions. Designer antibodies that genetically combine the rodent antigen-binding regions to human constant regions can significantly reduce the immunogenicity of the antibodies and may increase the efficacy of their effector functions as well (for recent reviews, see Austin, 1989; Mayforth and Quintans, 1990; Waldmann, 1991; Winter and Milstein, 1991). Humanized or chimeric antibodies should prove particularly useful when repeated administration of the antibody (as in treating chronic or recurrent diseases) is necessary. Other genetically engineered antibodies are also discussed in this chapter, including recombinant single-chain Fv fragments, V_H domains, antigen-binding peptides, and recombinant antibody fusion proteins. Much attention on the therapeutic use of antibodies in humans has focused on immunotoxins and anti-idiotypic antibodies. These antibodies can be (but are not necessarily) genetically engineered and merit the more extensive discussion that they receive in Chapters 4 and 5, respectively.

Genetically Engineered Rodent–Human Antibodies

Chimeric Antibodies

Genetic engineering has been used to create chimeric immunoglobulins by (1) combining the rodent V_H gene segment with human heavy-chain

constant region gene segments to make the heavy-chain gene construct, (2) connecting the rodent V_L gene segment with a human C_L exon to create the light-chain gene construct, and (3) transfecting both the heavy- and light-chain gene constructs into a nonsecretor myeloma (see Fig. 3.1) (Morrison *et al.*, 1984; Jones *et al.*, 1986; Verhoeyen and Reichmann, 1988). In principle, any rodent variable domain can be paired with any human constant region isotype so that the optimal combination of antigenic specificity and effector functions (such as complement fixation and ADCC (antibody-dependent cell-mediated cytotoxicity) can be selected (Morrison, 1985; Duncan and Winter, 1988). If necessary, fine-tuning of the constructs can be accomplished by introducing point mutations in the variable region gene segments that alter the affinity of the chimeric antibody for its ligand. For these reasons, molecular manipulations and transfectoma technology are popular means to "humanize" rodent antibodies with interesting specificities. These rodent–human chimeric antibodies are expected to be less antigenic and more useful in human therapy (Co *et al.*, 1991).

Different human heavy-chain isotypes can be chosen for the humanized antibody, depending on the desired therapeutic effect. For instance, when destruction of the target cell is desired, the human IgG1 constant region is often chosen since it is recognized by FcγR I, FcγR II, and FcγR III and mediates ADCC (see Tables 1.7 and 3.1) (Anderson and Looney, 1986). Human IgG3 also binds these FcγRs but has a much shorter serum half-life than IgG1. Also, although IgG3 is overall the most effective isotype at complement-mediated lysis, IgG1 is more effective at activating complement when high concentrations of the antigen are expressed on the target cell (Michaelsen *et al.*, 1991) (see Table 3.1). [Actually, some investigators have reported that the choice of the target antigen appears to be a more important factor in influencing complement-mediated cell lysis than choice of the antibody's isotype (Bindon *et al.*, 1988).] In other situations, such as in diagnostic imaging, antibody-receptor blocking, or antibody-mediated drug delivery, isotypes that bind Fc receptors poorly (or not at all) and/or are relatively ineffective at complement-mediated lysis (such as IgG2, IgG4, or IgA) may be the isotypes of choice.

The use of rodent monoclonal antibodies in humans can significantly alter the potency of their effector functions (see Table 3.1; for comparison, see Table 1.6 for the reactivity of murine IgGs with murine effector cells). One study reported that the FcγR III on human NK cells or lymphokine activated killer cells (LAK cells) was not able to mediate ADCC with mouse IgG1, IgG2a, or IgG2b antibodies (Dearman *et al.*, 1988). Other laboratories have reported the following relative isotype reactivity for ADCC with murine IgGs and human effector cells: murine (mu)IgG3 > muIgG2a > muIgG2b >> muIgG1 (not reactive) (Anasetti *et al.*, 1987;